Dealing with hypobaric hypoxia in the Andes: Genotypic Adaptation and Phenotypic Plasticity in the Torrent Duck (*Merganetta armata* Gould 1842)

Daniela Wilner[1*], Luis Alza[1,2], Emil Bautista, Natalia Gutiérrez-Pinto, María Lozano Jaramillo, Joana Projecto-Garcia, Cynthia Sánchez Figueroa, Matthew Smith, Jay Storz, Roy Weber, Kevin McCracken[1]

[1]Department of Biology and Wildlife, University of Alaska Fairbanks; [2]Centro de Ornitología y Biodiversidad (CORBIDI), Perú; *dwilner@alaska.edu

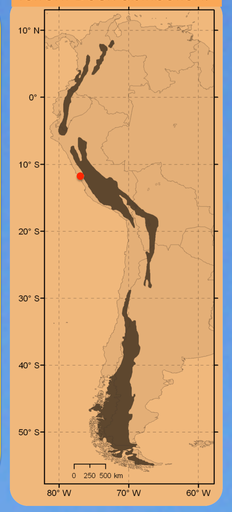
- Small (315 - 440 gr) and sexually **dimorphic** (in plumage and size).
- Reproductive season: **both parents care** for *young* (max. four ducklings).
- **Territorial** in reproductive season (min. one pair per Km).

Background

Torrent Ducks:

- **Unique**, diving, river specialists in South America,
- **Endemic** to whitewater Andean rivers,
- Found continuously across an altitudinal gradient from **900 to 4000 m** (Fjeldså and Krabbe 1990),
- Live across a variety of vegetational zones and climates,
- **Indicators** of river quality,
- **Vulnerable** and **declining** in parts of their range.
- Their habitat is under **threat** (Hilty and Brown, 1986), and human communities depend on the same rivers.

Torrent Duck distribution:

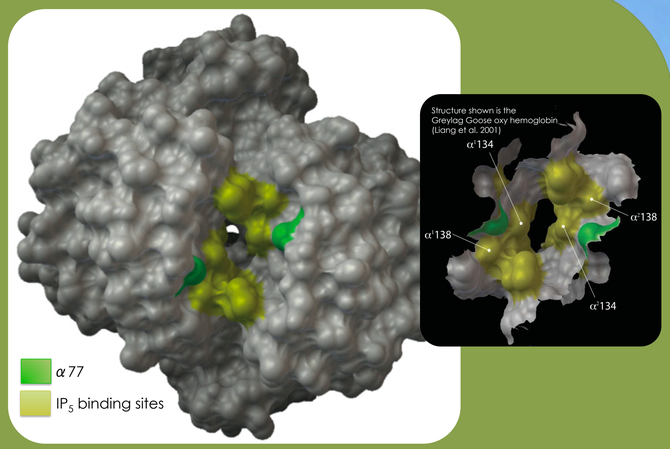


High Altitude Environment and Adaptations:

- Partial pressure of oxygen (PO_2) decreases with increasing altitude, and at 4000 meters it is approximately 60% of that at sea level.
- **Hypobaric hypoxia** (low partial pressure of oxygen at high altitude) is a major challenge for vertebrates, especially birds, and particularly when combined with low temperatures (Storz et al. 2010).
- **Phenotypic Plasticity**: When **lowlanders** experience high altitude hypoxia, they **acclimate** by **increasing their hemoglobin (Hb) concentration and their hematocrit** (proportion of red blood cells in blood). This allows them to deal with the reduced PO_2, but it puts stress on their circulatory and respiratory systems (Storz et al. 2010).
- Many species that **are adapted to high-altitude** have **low Hb concentration and hematocrit** (Hct) and different adaptations that allow them to live in the hypoxic environment, such as functional mutations in their Hb (**genotypic adaptation**). (Storz et al. 2010)

In **Torrent Ducks**, **two different alleles** can be found at **position α77 of their hemoglobin**: the **ancestral allele** codes for **alanine (Ala)**, and the **derived** allele codes for **threonine (Thr)**. This substitution was shown to have evolved in parallel in five lineages of high-altitude waterfowl (McCracken et al. 2009).

McCracken et al. (2009) predicted that **Thr-α77** might reduce phosphate (IP_5) sensitivity (IP_5 is an allosteric modulator that reduces Hb affinity for O_2) and shift the oxygen-hemoglobin dissociation curve to the left (**increasing Hb-O_2 affinity**).



Our question: What are Torrent Ducks doing to deal with hypobaric hypoxia? How are genotypic adaptation and phenotypic plasticity of hemoglobin interacting to allow them to survive in this harsh environment, across such a gradient of conditions?

Methods

Two field seasons (in 2011 and 2012) in Chillón river (●), Lima, Peru.

We captured **109 individuals** total (24 females, 87 males, 1 juvenile), using mist nets set across the river at several points over an altitudinal gradient **from 1041 to 4009 m**.

From each individual, we:
- Recorded sex and location of capture (including elevation).
- Extracted 3 ml of whole blood and stored it in liquid N_2 for genetic analysis.
- Quantified **hematological traits**:
  - **Hematocrit**:
    We collected blood in a 50 µl heparinized capillary tube (four replicate samples per individual) and centrifuged it in a ZIPocrit Portable Hematocrit Centrifuge (LW Scientific), then measured the proportions of packed red blood cells and plasma to calculate hematocrit.
  - **Hemoglobin Concentration**:
    We measured it using a Hemocue Hb 201 photometer (Hemocue Inc.) that measures azidemethemoglobin absorbance at 570 and 880 nm (Simmons and Lill 2006).

Statistical Analysis:
Simple linear regression analysis (phenotypic plasticity, reaction norm) fitted using the least squares approach between Hb concentration as the dependent variable and elevation as the independent variable, and Hb genotypes as two different treatments.

Results and Discussion

Following McCracken et al.'s (2009) prediction, we expected oxygen dissociation curves in Thr-α77 individuals to be left-shifted compared to Ala-α77, but surprisingly, we found **both curves** to be **left shifted**, and we found **no significant difference in Hb-O_2 affinity** between the two hemoglobin variants (Figure 1).

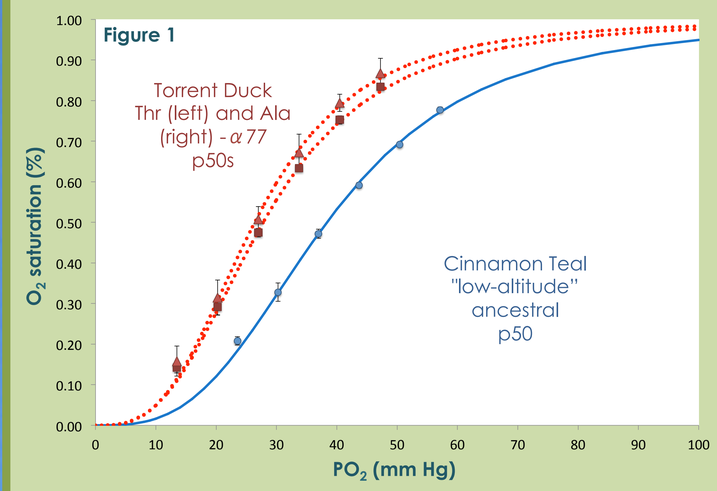
However, in seeming contradiction with these results, we did find a **stable interannual cline in Thr-α77 allele frequency**, by which said frequency **increased with elevation** (Figure 2).

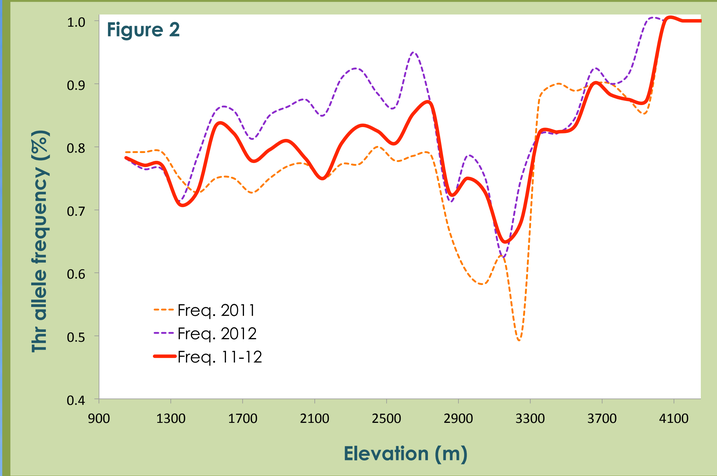
Furthermore, **hemoglobin** (Hb) **concentration** (Figure 3) **and hematocrit** followed the same **increasing trend with elevation**, showing a significant positive association between Hb concentration and elevation, with no association for the Ala/Ala genotype ($P = 0.332$), and a positive association for the Thr/Thr genotype ($P = 0.00117$).

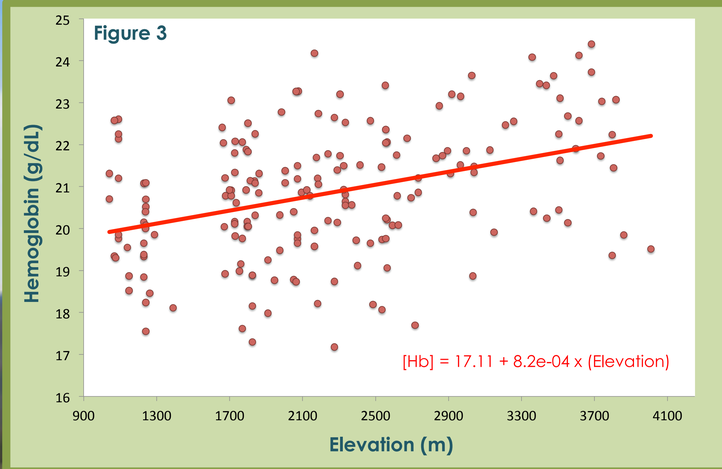
Lastly, individuals that were **homozygous** for the **Thr-α77** allele had significantly **lower Hb concentrations than homozygotes for the Ala-α77**, and **at high elevation**, they also appeared to **have lower Hb concentration than the heterozygotes**, **suggesting that the Thr-α77 allele is granting the individuals that possess it a mechanism that allows them to maintain lower Hb concentration at high elevations and is therefore being selected for in those environments**.

Based on these patterns, despite the lack of evidence for a difference in intrinsic or cofactor Hb-O_2 affinity in Thr-α77 and Ala-α77 variants, we believe that this **polymorphism may be of functional significance**. Perhaps the ex vivo tests misidentified the target of selection, or it may be that the experiment was too simplistic to reveal the complex physiological processes occurring in vivo, and there may also be epistatic interactions that are yet unaccounted for.

Torrent Duck p50s shown for stripped Hb & KCl + IHP







Future Directions:
- Sample more rivers in the area.
- Test a passerine bird that has the same habitat and range: White-capped Dipper (*Cinclus leucocephalus*).

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